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*Published in:*  
Anaerobic Digestion Conference AD16

*Publication date:*  
2019

[Link to publication from Aalborg University](#)

### *Citation for published version (APA):*

Lund Nielsen, J., Pedersen, N. K., Peydaei, A., Baudu, E., Fernando, W., Gurevich, L., Fojan, P., Wimmer, R., & Jonge, N. D. (2019). Potential for biodegradation of microplastics in thermophilic anaerobic digesters. In *Anaerobic Digestion Conference AD16*

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## Potential for biodegradation of microplastics in thermophilic anaerobic digesters

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### Abstract

Microplastics have emerged as new environmental pollutants. In this study biodegradation of polypropylene under thermophilic conditions were observed to be several orders higher than in any other environment investigated previously. A series of imaging AFM and SEM revealed presence of oxidation products accumulated on the surface, while spectroscopic analysis of the extracted and concentrated products were analysed by FTIR, Raman and NMR. The spectroscopic analysis revealed important insight into the composition, and a model of the polymer breakage involving formation of integrated cis-configured double bonds (olefins). The findings show a high potential for biodegradation of (micro)plastic in conventional ADs. The results furthermore support the use of applying anaerobic digesters for the treatment of highly energetic household waste with a high content of plastics.

**Keywords: microplastic; biodegradation; microbial community**

*Session – Anaerobic biotransformation of micropollutants*

### Introduction

Microplastics (MP) are a growing global pollutant in the water infrastructure, stemming from larger plastic waste as well as industrial products containing additives such as microbeads. Common plastic types include polyethylene (PE), polystyrene (PS) and polypropylene (PP) (Ziajahromi *et al.*, 2017). At the endpoint of domestic water usage, microplastics enter the wastewater treatment facilities, where some, but not all are removed with the surplus sludge, causing the MP to be released into the environment (Estahbanati and Fahrenfeld, 2016)). Surplus sludge is often further degraded in anaerobic digesters (AD) and thereafter used as agricultural fertilizers. The AD system contains a complex microbial community which has previously been shown to biodegrade a diverse range of xenobiotic compounds (Gonzalez-Gil *et al.*, 2016). The present study aimed to investigate the potential biodegradation of polypropylene in anaerobic digesters.

### Materials and Methods

#### *Degradation assay of PP at lab-scale*

Polypropylene plastic beads (3 mm) or coupons (1 cm<sup>2</sup>) were incubated in 1L reactors containing 750mL fresh collected biogas slurry, from Aalborg West wastewater treatment plant, under realistic thermophilic AD conditions. Samples of PP and digester slurry were collected at day 0, 2, 4, 8 and 12.

### *Surface analysis of PP*

The molecular changes to the surface of PP over 12 days of incubation were analysed using FTIR and Raman microspectroscopy. Changes to the PP surface were further visualized using Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). Surface material from the PP beads was collected by partially dissolving the beads in 100% chloroform for 3h at room temperature. Extracted surface PP were lyophilized and redissolved in 600  $\mu\text{L}$   $\text{CDCl}_3$  (Eurisotop, 99%D), including 0.03% TMS and analyzed on a BRUKER AVIII-600 MHz Nuclear Magnetic Resonance (NMR) spectrometer. The following spectra were recorded:  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR, [ $^1\text{H}$ ,  $^{13}\text{C}$ ]-HSQC (multiplicity-edited and sensitivity-improved), HMBC, HSQC-TOCSY, DQF-COSY and DOSY.

### *Microbial community analysis*

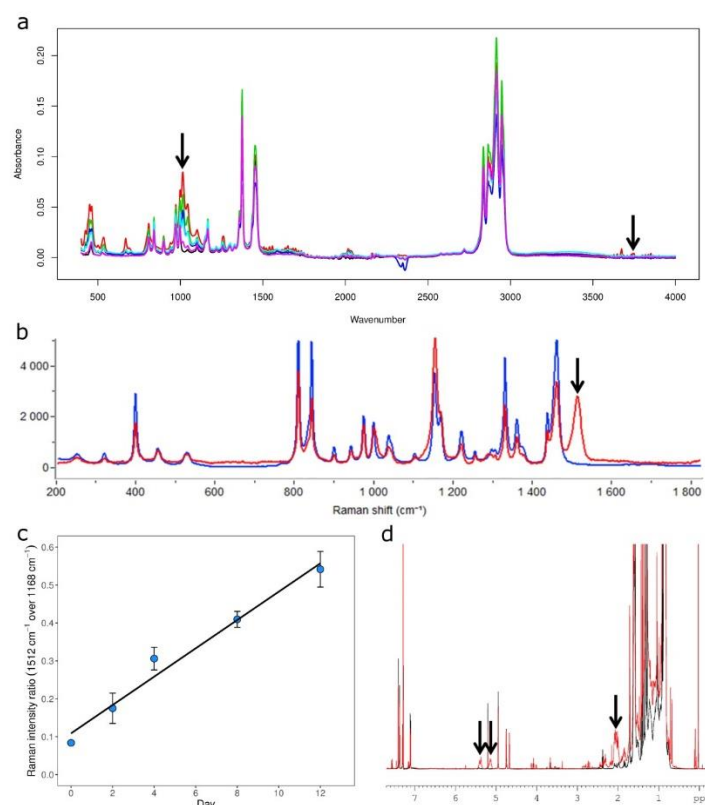
Total DNA was extracted from AD slurry and PP coupons, and the V4 hypervariable region of the 16S rRNA gene was sequenced on an Illumina Miseq platform, and analysed using multivariate statistics and visualized in R.

## **Results and Conclusions**

After incubation under thermophilic AD conditions, the surface characteristics of the PP coupons were analysed using spectroscopic methods (Figure 1). Analysis with FTIR ( $n = 5$ ) showed changes to the composition of the plastic surface (Figure 1b), primarily potential oxidation damage. Further analysis with Raman microspectroscopy revealed an additional Raman active peak at  $1512\text{ cm}^{-1}$  (Figure 1a), supporting the evidence of surface changes as well as the appearance of degradation products. Comparison of the  $1512\text{ cm}^{-1}$  against an unchanged marker of PP revealed a linear increase of the new peak over the course of 12 days (Figure 1c). NMR analysis of extracted PP surface compounds supported the appearance of new oxidations products after 12 days of incubation. Spectral analysis by Raman and the NMR suggested the formation of an oxidation product containing C=C bonds. This double bond could be identified as being part of the polymer backbone forming an integral, cis configured polyisoprene-like structure. Furthermore, NMR analysis revealed significant reduction in the polypropylene chain length.

To characterize the degradation of PP in more detail, the surface of the PP coupons was visualized using Raman microspectroscopy mapping (Figure 2). Mapping of surface squares confirmed changes, in the form of approx.  $30\mu\text{m}$  large formations of oxidation products (Figure 2a and 2b).

Further visualization with AFM microscopy (Figure 2c and 2d) showed a decrease in the roughness of the PP surface after 12 days, suggesting that the surface of the plastic had become smoother. Imaging with SEM microscopy revealed the presence of biofilm formation with a high content of filamentous bacteria, but also microbes of other morphologies. Pitting was observed in proximity to the microorganisms, while the general surface smoothening confirmed the observations by AFM.

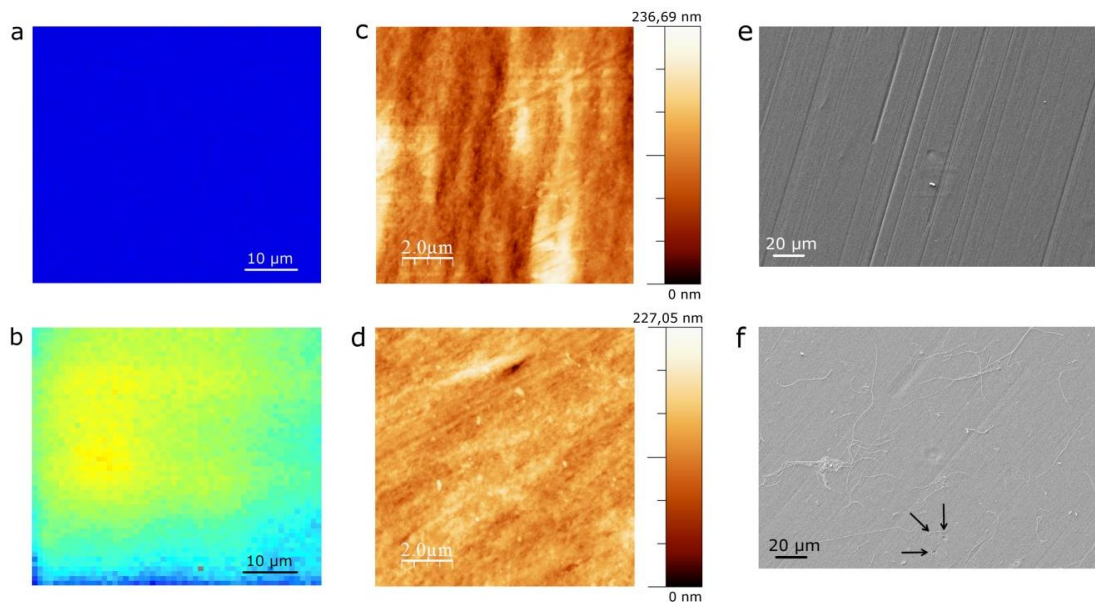


**Figure 1: Detection of polypropylene degradation and associated oxidation products using FTIR (a), Raman microspectroscopy (b) and quantification (c), and NMR (d).**

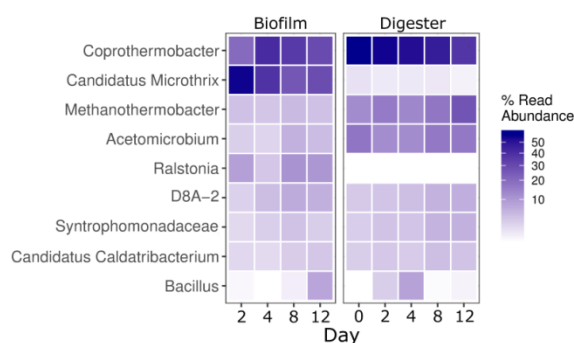
To characterize the microbial community composition of the AD slurry as well as the biofilm on the PP coupons, 16S rRNA gene amplicon sequencing was performed (Figure 3). The community structure was observed to change between digester and PP samples, supporting the evidence that biofilm is formed on the PP over the course of 12 days. Organisms that were observed almost exclusively in the biofilm samples included representatives of *Candidatus* Microthrix and *Gordonia*, both of which are known filamentous microorganisms frequently observed in WWTPs. Interestingly, both of these filamentous organisms have been regarded as strict aerobic organisms and their presence in AD has previously been regarded as relative persistent immigrants deriving from the WWTP sludge used as substrate (Kirkegaard *et al.*, 2017).

Preliminary experiments indicate that the newly observed biodegradation of polypropylene appears to be highly temperature dependent and thus more significant in thermophilic than mesophilic digester systems (data not shown).

In conclusion, the results of the present study suggest that polypropylene is degraded in digesters under thermophilic conditions. The changes to the overall surface of the plastic, as well as the formation of biofilm suggest a combined mode of corrosion and biodegradation by microbes. This study is the first direct evidence of microplastic degradation in AD systems, and highlights the potential for MP removal as an alternative mode of operation for digesters at wastewater treatment facilities.



**Figure 2: Visualisation of surface changes to PP before and after incubation, using Raman mapping (a and b), AFM microscopy (c and d) and SEM microscopy (e and f).**



**Figure 3: Heatmap of the microbial community compositions in the biofilm formed on polypropylene and the digester slurry after 2, 4, 8 and 12 days of incubation in thermophilic digester sludge.**

Furthermore, it can also be speculated that anaerobic digestion of waste containing high amount of plastics, such as food waste, might hold potential for removal or partial transformations. Exploration of such a bioplastic degradation potential hold great promises for future applications of the AD technology.

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